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Chapter 4

Tryptophan depletion and serotonin release - a critical reappraisal

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Abstract

Tryptophan depletion is often assumed to lead to a decrease in neuronal serotonin release in the brain. Here we review the literature and show that only in animal studies in which either serotonin synthesis rate is already decreased or serotonin utilization is increased an effect of tryptophan depletion on serotonin release has been demonstrated. In the absence of convincing evidence for reduced central serotonin release, the possibility that other mechanisms are involved cannot be discarded. We therefore conclude that one should be careful to interpret tryptophan depletion-related effects as reflecting a widespread reduction of central serotonin release.

Introduction

Tryptophan depletion was introduced about 30 years ago as an experimental method to obtain a rapid and reversible decrease of the concentration of serotonin in the brain (Biggio et al., 1974; Gessa et al., 1974). By ingestion of a mixture of amino acids without the serotonin precursor tryptophan, the rate of serotonin synthesis in the brain is strongly decreased (Nishizawa et al., 1997). Since its introduction in human psychopharmacology (Young et al., 1985), this method has been used increasingly as a relatively simple and safe method to assess serotonergic functions. A PubMed search delivers about 50 articles per year for 'tryptophan depletion', and the popularity of the method appears still to be increasing.

Most authors tacitly assume that tryptophan depletion will reliably and reproducibly decrease serotonin transmission because of its effect on synthesis. However, this is only possible if serotonin release and binding to postsynaptic receptors is affected, as well. While some authors indeed mention this explicitly, the actual evidence is extremely sparse and often misquoted. Therefore, we will review what evidence is available to support that tryptophan depletion affects serotonin release and serotonin receptor binding.

Tryptophan depletion

The rate-limiting step in the synthesis of serotonin is the hydroxylation of tryptophan to 5-hydroxytryptophan by tryptophan hydroxylase, the enzymatic marker of serotonin neurons. This means that synthesis is directly dependent on the supply of the precursor, tryptophan. Free plasma concentrations of this essential amino acid are determined by its intake, plasma protein binding and utilization in a number of physiological processes, of which serotonin synthesis is only one. Tryptophan can enter the brain through active uptake by a carrier shared with other large, neutral amino acids. Intake of a tryptophan-free diet (Biggio et al., 1974) or a mixture of essential amino acids (Gessa et al., 1974) leads to increased demand for tryptophan for protein synthesis and decreased brain uptake. Blocking protein synthesis indeed prevents the effect on brain uptake (Moja et al., 1991). Thus, plasma and brain concentrations of tryptophan are strongly decreased, and this leads to decreased concentrations of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the brain tissue (Biggio et al., 1974; Gessa et al., 1974). Whether this decrease will have consequences for serotonergic neurotransmission depends on the utilization (i.e. the net loss) of the transmitter because of activity-dependent release and metabolism. If utilization is too high in comparison to the reduced synthesis, releasable pools of serotonin will be exhausted and serotonergic function will be affected.

Numerous physiological, pharmacological and behavioral experiments have been carried out after tryptophan depletion, and these have been reviewed regularly (Delgado et al., 1989; Young and Teff, 1989; Reilly et al., 1997; Fadda, 2000; Moore et al., 2000; Bel et al., 2001; van der Does, 2001; Riedel et al., 2002; Booij et al., 2003; Fusar-Poli et al., 2006; Evers et al., 2007; Ruhé et al., 2007). An important issue is that tryptophan depletion is often used to prove that a function is dependent on serotonergic activity or not – i.e. as an 'acid test' for serotonergic involvement. We feel that the evidence for this is not strong enough. For a reliable use of the test, unequivocal evidence should be available that tryptophan depletion reproducibly decreases serotonin release and serotonergic transmission. We will argue that this is not the case, and that the effects strongly depend on the precise experimental conditions. We will review the evidence that tryptophan depletion affects serotonergic neuronal activity, serotonin release and serotonin receptor activation in the following sections.

Effects of tryptophan depletion on 5-HT release and neuronal activity

The firing activity of serotonergic neurons in the raphe nuclei is subject to serotonergic control through autoreceptors. Increases or decreases in extracellular serotonin from dendritic or collateral release are reflected in decreased or increased neuronal activity, respectively. Early studies showed that the former effect, decreased neuronal activity,

was indeed consistently observed after tryptophan administration (Aghajanian, 1972; Trulson and Jacobs, 1975). A tryptophan-free diet, however, did not significantly change firing in cats, even though brain tryptophan, serotonin and 5-HIAA concentrations were decreased (Trulson, 1985).

The same author showed that serotonin release measured by push – pull perfusion was not affected in this condition, although the concentrations of 5-HIAA in the perfusate were decreased. Later studies of extracellular serotonin concentrations used microdialysis sampling, which is now the method of choice to determine serotonin neuronal release (Westerink, 1995). These studies are summarized in Table 1. Our conclusion is that serotonin release is only reduced by tryptophan depletion when the diet is chronically administered, when a serotonin reuptake inhibitor is used, or when serotonin release is strongly stimulated. The effect of chronic administration of the diet is clearly illustrated by the results of Fadda and colleagues (Fadda, 2000; Fadda et al., 2000a, 2000b), who showed that administration of the diet for 3 – 5 days leads to steadily decreasing extracellular levels of serotonin in rat hippocampus and frontal cortex. Chronic administration of such diets is also known to affect body weight (see, for example, D'Souza et al., 2004 ; Cahir et al., 2007) and plasma corticosterone levels (D'Souza et al., 2004). It cannot be expected that the physiological or neurochemical alterations after long-term diet administration are exclusively dependent on serotonergic mechanisms.

Addition of serotonin reuptake blockers to the dialysis fluid was often applied in older studies to increase the extracellular concentrations, and therefore the detectability of serotonin in the dialysates. However, it has long been known that reuptake blockade leads to decreased serotonin synthesis rates (Carlsson and Lindqvist, 1978), and this will increase the chances that synthesis cannot keep up with utilization when the tryptophan availability is reduced.

The effect of uptake inhibition is clearly demonstrated in the set of experiments reported by Bel and Artigas (1996), who studied the effects of peripheral administration of the uptake blocker fluvoxamine. Only after chronic fluvoxamine did tryptophan depletion lead to decreased serotonin release in the dorsal raphe nucleus or frontal cortex; after saline treatment, no effect was observed. Fasting or feeding the rats before the experiment did not alter the fluvoxamine effect.

Gartside et al. (1992b) also reported no effect on basal release of tryptophan depletion by treatment with one of the essential amino acids that competes with tryptophan, i.e. valine. However, they observed a reduced effect of electrical stimulation of the raphe nucleus or administration of fenfluramine, resulting in two-fold and sixfold increases of release in control animals, respectively (Gartside et al., 1992a, 1992b). It would be interesting to test whether normal, physiological activation of serotonergic activity would also uncover the effects of tryptophan depletion on *in vivo* serotonin release.

Table 1. Effects on serotonin release.

Release	Measurement	Subjects	Method	Biochemical effect	Effect on 5-HT release	Reference
push-pull perfusion (^{3}H-trp)	lateral ventricle	cat	fast; trp+ or - diet	- 49% trp; -27% 5-HT tissue	no signif effect	Trulson, 1985
in vitro slice (fluoxetine)	hypothalamus	rat	trp+ or – superfusion	+ or – tissue trp, 5-HT	+ or – basal & evoked	Schaechter & Wurtman, 1990
microdialysis (citalopram)	frontal cortex	rat; anesth	AA-mix oral	- 61% trp; - 39% 5-HT tissue	- 30% basal	Heslop et al., 1991
microdialysis (citalopram)	ventral hippocampus	rat	AA-mix ip inj	not reported	- 32% raphe stimulation - 20% fenfluramine effect	Gartside et al., 1992a
microdialysis (citalopram)	ventral hippocampus	rat; anesth	valine ip inj	not reported	- 40% fenfluramine effect	Gartside et al., 1992a
microdialysis (citalopram)	ventral hippocampus	rat; anesth	valine ip inj;	not reported	no effect basal - 53% raphe stimulation	Gartside et al., 1992b
microdialysis (chron fluvox sc)	frontal cortex, d-raphe	rat	fast; AA-mix oral	not reported	- 50% basal d-raphe - 39% basal frontal cortex	Bel & Artigas, 1996
microdialysis	frontal cortex, d-raphe	rat	AA-mix oral	not reported	no signif effect	Bel & Artigas, 1996
microdialysis (chron fluvox sc)	frontal cortex, d-raphe	rat	AA-mix oral	not reported	- 68% basal frontal cortex	Bel & Artigas, 1996
microdialysis (citalopram)	dorsal hippocampus	rat	fast; AA-mix oral	not reported	- 35% basal - 62% fenfluramine effect	Stancampiano et al., 1997a
microdialysis (citalopram)	cortex, d.hippocampus	rat	fast; trp-free diet	not reported	- 30% cortex - 50% hippocampus	Stancampiano et al., 1997b
microdialysis (citalopram)	frontal cortex	rat	fast; trp-free diet	not reported	no effect (day 1); - 75% (day 3)	Fadda et al., 2000b
microdialysis (alaproclate)	frontal cortex	rat	fast; trp-free diet	not reported	- 40% (day 1); - 75% (day 3)	Fadda et al., 2000a
microdialysis	dorsal hippocampus	rat	low or high trp diet	-20%; no effect	- 44% (day 4); + 92% (d 7)	v.d.Stelt et al., 2004
microdialysis	medial PFC	rat	fast; gelatin oral	-71% plasma trp	no signif effect	v.d.Plasse et al., 2007a

Abbreviations: AA - amino acid; anesth - anesthesized; chron - chronic; d - dorsal; fluvox - fluvoxamine; ip - intraperitoneal; sc - subcutaneous; signif - significant; trp – tryptophan.

Van der Plasse et al. (2007a) exposed rats to a novel environment and measured release after administration of the tryptophan-free diet. However, this stimulus was apparently too weak to activate serotonin release. More conclusive evidence might be obtained by combining tryptophan depletion with studies of in vivo release under conditions of more consistently increased serotonergic activity, such as motor activity (Jacobs and Fornal, 1997) or stress (Amat et al., 2005).

A further issue that has not been taken into account is the differential innervation of brain areas by the median and dorsal raphe nuclei. These form two distinct serotonergic systems, with different morphological and pharmacological properties (Molliver, 1987). As differences have been shown to exist in stress-induced serotonin release and the effects of chronic uptake inhibition in these systems (Auerbach and Hjorth, 1995; Storey et al., 2006), it would be interesting to study their response to tryptophan depletion.

In conclusion, only in conditions of enhanced serotonin utilization or reduced serotonin synthesis has an effect of tryptophan depletion on serotonin release been demonstrated.

Direct measurements of serotonin extracellular concentrations in primate brain tissue have not been reported, but a few studies were performed using cerebrospinal fluid (CSF), which is in direct contact with the extracellular fluid of the brain and spinal cord.

All studies showed profound decreases of tryptophan concentrations in plasma, CSF or brain tissue, and furthermore reported 5-HIAA decreases of 30 percent (Table 2). No study, however, reported on serotonin concentrations. The value of 5-HIAA measurements is limited, as extracellular concentrations of 5-HIAA cannot be expected to be a reliable indicator of neuronal serotonin release (Crespi, 1990). Deaminated monoamine acidic metabolites are not stored in the neuron, like the parent transmitter, but freely exchange between intra- and extracellular spaces. Their concentration is determined by the metabolism of released, but also of newly synthesized, transmitter, and by active transport out of the brain by the probenecidsensitive carrier (Westerink, 1995). While extracellular 5-HIAA may show changes similar to those of serotonin, this is not necessarily the case, and differential effects may be expected when either uptake or synthesis is altered (Stenfors and Ross, 2004; van der Stelt et al., 2005). In summary, alterations in CSF 5-HIAA levels cannot be taken as proof of altered serotonin release.

Another outcome of all these studies is that a profound reduction of plasma tryptophan, the usual measure of the effectiveness of tryptophan depletion in human studies, is not necessarily associated with decreased *in vivo* serotonin release in the brain. A clear example is provided by van der Plasse et al. (2007a), who showed strongly decreased plasma tryptophan in the absence of a significant alteration of rat-

brain serotonin release. While this should be confirmed in other studies, it supports the conclusion that a central imbalance between serotonin synthesis and utilization is needed to observe an effect of tryptophan depletion.

Effects of tryptophan depletion on serotonin receptors

An alternative approach to study the effects of tryptophan depletion on serotonin release is to determine *in vivo* occupation of serotonin receptors or serotonin receptor mediated effects or *ex vivo* receptor numbers. An overview of the few studies that have been performed is presented in Table 3. Decreased serotonin release should be reflected as an increase in radioligand binding potential. This has not been reported. Human PET studies of radioligand binding to 5-HT_{1A} receptors after tryptophan depletion did not detect any alteration, which is remarkable, as a study of the effects of inhibition of tryptophan hydroxylase on 5-HT_{1A} binding in rats using the same radioligand (18 F-MPPF) did report the expected clear increase in binding (Zimmer et al., 2003). The only study of 5-HT 2 receptors did not detect an increase, but rather a decrease, in binding potential (Yatham et al., 2001).

Animal studies on receptor-occupancy or receptor mediated effects are sparse, too (Table 3). Moreover, most of the results were obtained after chronic tryptophan-free or low-tryptophan diets (and not all these experiments are reported here). The only acute effect is a decrease in 5-HT_{1A} receptor numbers in the raphe nucleus (Cahir et al.,2007), which again cannot be explained by a reduction in occupancy by serotonin. Instead, the authors suggest a homeostatic response to reduce autoreceptor feedback, and they discuss the possibility that alterations in corticosterone release might mediate this effect. Others, however, conclude that serotonin is only involved in cortisol/corticosterone release in response to stress, but not under basal conditions (Porter et al., 2007).

Thus, none of these studies provides any evidence of decreased serotonin release after tryptophan depletion.

Other effects of tryptophan depletion – alternative mechanisms

As was mentioned previously, tryptophan is used in many physiological processes, both peripherally and centrally. While the possible consequences of a reduction of central serotonin synthesis receive most attention, it cannot be excluded that other processes are involved in the physiological or behavioral effects that have been reported after tryptophan depletion. Therefore, we need to consider first whether serotonergic or non-serotonergic peripheral processes might be involved in these effects, and second, whether non-serotonergic central processes might play a role.

Table 2. Measurements in primates.

Release, synthesis	Measurement	Subjects	Method	Biochemical effect	Effect on 5-HT	Reference
CSF concentrat.	cisternal CSF	vervet monkey	fast; AA mixture	CSF - 61% trp; - 34% 5-HIAA	not measured	Young et al., 1989
5-HT synthesis	human PET; ^{11}C-Me-L-Trp	volunteers	diet; AA-drink	plasma - 72% trp; synth rate - 87% male; plasma - 89% trp; synth rate - 97% female	not measured	Nishizawa et al., 1997
CSF concentrat.	lumbar CSF	volunteers	diet; AA-drink	plasma - 89% trp; CSF - 92% trp; - 31% 5-HIAA	not measured	Carpenter et al., 1998
CSF concentrat.	lumbar CSF	volunteers	diet; AA-drink	plasma - 86% trp; CSF: - 92% trp; - 33% 5-HIAA	not measured	Williams et al., 1999

Table 2. Abbreviations - AA - amino acid; CSF - cerebrospinal fluid; Me-L-trp - α-methyl-L-tryptophan; PET - positron emission tomography; synth – synthesis.

Table 3. Receptor measurements.

Receptor	Receptor measurement	Subjects	Method	Biochemical effect	Effect on receptor	Reference
5-HT_{1A}	human PET; ^{18}F-MPPF	volunteers	diet; AA-drink	- 62% plasma trp	no signif eff	Udo de Haes et al., 2002
5-HT_{1A}	human PET; ^{18}F-MPPF	remitted patients	AA-drink	- 85% plasma trp	no signif eff	Prashak-Rieder et al., 2004
5-HT_{1A}	human PET; 18F-FCWAY	volunteers	not reported	not reported	no signif eff	Williams et al, 2002
5-HT_{2}	human PET; ^{18}F-setoperone	volunteers	AA drink	- 71.5% plasma trp	- 7.9% BP cortical areas	Yatham et al., 2001
5-HT_{1A}	blood hormones	rat	3 week trp-free diet	- 41% 5-HT FCo	- 40% OXT resp agon - 69% ACTH resp agon - 100% corticost resp agon	D'Souza et al., 2004
5-HT_{1A}	rec binding brain	rat	acute or chron diet	- 80% plasma trp; - 30-80% brain 5-HT	acute: - 14% raphe rec number	Cahir et al., 2007
5-HT_{1A}	prolactin resp; rec binding brain	rat	1 week low-trp diet	- 30-40% plasma trp - 20% cortex 5-HTP	+ 200% 2 min resp agon no signif eff rec number	Franklin et al., 1999
5-HT_{2A}	rec binding brain	rat	acute or chron diet	- 80% plasma trp; - 30-80% brain 5-HT	3 week: + 46% cortex rec number	Cahir et al., 2007
5-HT_{2C}	prolactin resp; rec binding brain	rat	6 weeks low-trp diet	- 40-60% plasma trp - 43% cortex 5-HTP	+ 136% AUC resp agon no signif eff rec number	Franklin et al., 1999

Table 3. Abbreviations (next page): AA - amino acid; ACTH - adrenocorticotroph hormone; agon - agonist; AUC - area-under-the-curve; BP - binding potential; chron - chronic; corticost – corticosterone; FCo - frontal cortex; FCWAY - Trans-4-Fluoro-N-(2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl)-N-(2-pyridyl)cyclohexanecarboxamide; MPPF - (4-(2'-methoxyphenyl)-1-[2'-(N-2"-pyridinyl)-p-fluorobenzamido]-ethyl piperazine); OXT - oxytocine; PET - positron emission tomography; rec - receptor; resp - response; signif eff - significant effect; trp - tryptophan;

Peripheral processes

Despite the fact that only 5 percent of the total amount of serotonin present in the body is found in the brain, and that serotonin receptors are distributed throughout the body (see, for example, Ramage and Villalón, 2008), relatively little is know about consequences of tryptophan depletion on serotonergic functions outside the brain. Serotonin has been implicated as an important modulator of cardiovascular and gastrointestinal activity. Although peripheral mechanisms can be expected to predominate, these processes may also partly depend on central serotonin availability. It is thus not surprising that tryptophan has been suggested to link somatic and psychiatric illnesses (Russo et al., 2003). The fact that most serotonin is synthesized and utilized peripherally supports such a notion.

Peripheral serotonin is synthesized in, and released from, the enterochromaffin cells of the intestinal system. It is known to be involved in the regulation of gastrointestinal activity (Kilkens et al., 2004), and tryptophan depletion affects gastric emptying in healthy female controls (van Nieuwenhoven et al., 2004). A potentially important observation is that tryptophan depletion in a control group and in patients with irritable bowel syndrome (IBS) not only impaired affective memory performance, but also heightened visceral perception. Shufflebotham et al. (2006) replicated these findings in patients with IBS, but not in healthy controls.

Central as well as peripheral actions of serotonin on cardiovascular output have been well described. In short, serotonin acts both directly via the raphe nucleus on (para) sympathetic innervation, and as a modulator on serotonergic receptors on the smooth muscle tissue of the heart and vascular system. As such, serotonin receptor activation has been shown to increase, as well as decrease, heart rate (Jonnakuty and Gragnoli, 2008), and to regulate blood pressure (Nilsson et al., 1999). The effect of tryptophan depletion on these variables remains, however, unclear. A 2-day dietary depletion of tryptophan enhanced stress-induced increases in blood pressure in recovered anxiety patients treated with serotonin-reuptake inhibitors (Davies et al., 2006), but Roiser et al. (2008) did not observe any change in blood pressure or heart rate after acute tryptophan depletion in healthy controls. A study by van der Veen et al. (2008) has, to the best of our knowledge, been the only one to find a correlation between tryptophan depletion and cardiac slowing, although the authors attributed this to an effect of task rather than a depletion of serotonin levels.

These examples are illustrative of the idea that perception of bodily functions (e.g. increased heart rate or gastrointestinal activation) affects cognitive appraisal – an idea that is certainly not new. The influential concept was originally posed in the nineteenth century by William James and Carl Lange, and later took the form of the somatic-marker hypothesis posed by Damasio (1996). This model, in essence, entails the notion that affective signals from the body are used to guide decision-making, and as such play an important role in the expression of behavior.

Although the exact nature of peripheral effects induced by (acute) tryptophan depletion is poorly understood, there are positive indications that tryptophan depletion does affect functions outside the brain that deserve consideration when interpreting the central effects of tryptophan depletion.

Central processes

In addition to possible effects of tryptophan depletion on peripheral processes, an important question is the extent to which tryptophan depletion might directly exert its effects through modulation of other central monoamines. Effects of tryptophan depletion might be expected, as the catecholamine precursor tyrosine is one of the amino acids that are administered and that compete for transport over the blood – brain barrier (Badawy, 2005). However, *in vivo* microdialysis studies showed that neither noradrenaline nor dopamine release was altered after tryptophan depletion in rats (Fadda et al., 2000a; van der Plasse et al., 2007a). In addition, brain concentrations of these neurotransmitters and their metabolites were not affected (Lieben et al., 2004b; Ardis et al., 2008). Even tyrosine concentrations were not consistently altered (Lieben et al., 2004b; Jans et al., 2007b).

Measurements in CSF indicated that tyrosine concentrations were not changed (Young et al., 1989) or increased (Carpenter et al., 1998) while, again, dopamine and noradrenaline metabolites were not altered. Therefore, there is no indication that tryptophan depletion leads to alterations in brain catecholamines that might explain the physiological and behavioral effects.

Effects of tryptophan depletion on non-serotonergic processes that use tryptophan have not been studied in great detail. However, Lieben et al. (2004c) performed a pilot study on the effect of tryptophan depletion on kynurenine-related metabolites in serum, liver and brain. An interesting finding was that tryptophan was decreased in serum and brain, but not in liver. In liver, only the concentration of quinolinic acid was decreased, but the brain concentrations of neither kynurenine, nor kynurenic acid or quinolinic acid were decreased (Lieben et al., 2004c), making it unlikely that tryptophan depletion affects central processes through this pathway.

Consequences for the interpretation of tryptophan-depletion induced effects

The greatest impact that the tryptophan-depletion method has made is undoubtedly in the field of psychiatry. Since the reports that tryptophan depletion may lower mood in normal males (Young et al., 1985) and in remitted depressed patients (Delgado et al., 1990), this method has been used to study serotonergic involvement in psychiatric disorders (Reilly et al., 1997; Bel et al., 2001; van der Does, 2001; Booij et al., 2003). Recently, attention has increasingly been directed to the use of tryptophan depletion

in cognitive research, as well (Park et al., 1994; Riedel et al., 1999; Riedel, 2004; Fusar-Poli et al., 2007). Tryptophan-depletion studies now form the basis for novel theories about the role of serotonin in behavior and cognition (Evers et al., 2007; Cools et al., 2008b).

However, before we accept the apparent use of tryptophan depletion as an ' acid test' for serotonergic involvement in human clinical and cognitive research, we have to return to our earlier conclusion that tryptophan depletion only decreases serotonin release and function in specific conditions – i.e. treatment with serotonin reuptake blockers, reduced serotonin synthesis and increased serotonin utilization. Recent clinical literature indeed shows that the reported effects of tryptophan depletion are not always reproducible. Indications that effects are observed only in special experimental conditions or only in subpopulations of either patients or normal controls abound in the literature. Thus, the effectiveness of tryptophan depletion as a method for studying serotonin involvement in mood is restricted to subpopulations that show a vulnerability to serotonin dysregulation (Fadda, 2000; Booij et al., 2003; Fusar-Poli et al., 2006; Jans et al., 2007a; Ruhé et al., 2007). These subgroups include remitted depressed patients (generally those treated with selective serotonin reuptake inhibitors; see Bremner et al., 1997; Young and Leyton, 2002; Neumeister et al., 2004), females (who show lower synthesis rates; see Ellenbogen et al., 1996; Nishizawa et al., 1997; Schmitt et al., 2000; Booij et al., 2002) and subjects with certain genetic variations in the serotonin transporter gene (associated with reduced serotonin reuptake; see Roiser et al., 2006; Finger et al., 2007). Based on the animal data, one might add that the level of activation due to different task demands and its interaction with the biological vulnerability will prove to be an important factor, as well.

The examples mentioned in the section on peripheral processes also suggest that the effects of tryptophan depletion probably depend on the individual vulnerability of the participants. The same should apply to the studies of animal and human cognition. Regarding the human studies, the issue is further complicated by the fact that many studies using tryptophan depletion report changes in brain activation measured by functional MRI, in the absence of effects on performance or mood (Fusar-Poli et al., 2007; Anderson et al., 2008). Given the uncertainty regarding underlying mechanisms, the inferences for serotonergic functions of these results remain tentative.

The possibility that individual differences in emotional processing and its underlying biological processes are a major factor in the variability of tryptophan-depletion induced effects on cognition has been raised (Evers et al., 2007; Harmer, 2008). Supportive evidence was recently provided by (1) the work of Sambeth et al. (2007), who found that impairments in memory recall after tryptophan depletion were stronger in females than in males, and (2) the work of Olivier et al. (2008), who tested object memory in rats with genetic deletions of the serotonin transporter. Tryptophan

depletion led to similar effects on plasma tryptophan in all genotypes, but differential effects on brain serotonin concentrations and object recognition.

Conclusion

The evidence that tryptophan depletion leads to a reduction of serotonin release and transmission is sparse, and is restricted to animal studies in which either serotonin synthesis rate is already decreased or serotonin utilization is increased. The variability in the results of human tryptophan depletion studies can tentatively be explained by taking the biological vulnerability and the task demands into account. However, in the absence of convincing evidence for reduced central serotonin release, the possibility that other mechanisms are involved cannot be discarded. We conclude that one should be careful to interpret tryptophan depletion-related effects as reflecting a widespread reduction of central serotonin release.